U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

CHAPTER 10

r-BIOPHARM RIDASCREEN®FAST DON TEST KIT

10.1 TESTING AREA

The extraction solution and other materials used in the r-Biopharm RIDASCREEN®FAST DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

10.2 EXTRACTION PROCEDURES

a. Place 50 grams of ground sample into a suitable container (e.g., plastic bag).

b. Add 250 ml of distilled/deionized water and seal/close container securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter the extract through Whatman #1 filters (or equivalent) into a clean container that is labeled with sample ID number.

f. Dilute the filtered extract with one part sample extract to 3 parts distilled/deionized water. (e.g., 1 ml sample extract plus 3 ml water)

g. Use 50 µl of the diluted filtrate per well in the test.

10.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water.

b. Swirl to mix.

When stored properly (at 39º F) the solution has a shelf life of four weeks.

10.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents and antibody wells to reach room temperature (68 - 77º F) prior to running the test.

(2) Insert a sufficient number and wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C .222	C .666	C 2.0	C 6.0	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C .222 is the 0.222 ppm control, C .666 is the 0.666 ppm control, C 2.0 is the 2.0 ppm control, and C6 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standards and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of deoxynivalenol antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 5 minutes (± 1 minute) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 3 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, Awareness Technology Stat-Fax Model 303 PLUS, or the Hyperion Microreader™ 3 Model 4027-002, microwell readers.

(Results must be read within 10 minutes)

b. Reading the Results.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

(3) Hyperion Microreader™ 3 Model 4027-002 Microwell Reader

(a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(b) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(c) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(e) The reader will read the optical density of the wells and print a report.

(f) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(g) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

10.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

10.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

10.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

10.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

10.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

(1) 1 microtiter plate.

(2) 48 Antibody coated wells.

(3) 5 DON standard solutions of 1.3 ml each; 0, 0.222, 0.666, 2.0, and 6.0 ppm DON in water.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated deoxynivalenol solution.

(5) 1 black-capped bottle of 3 ml anti- deoxynivalenol antibody.

(6) 1 white dropper bottle of 6 ml Substrate/Chromagen, stained red.

(7) 1 yellow or orange dropper bottle of Stop reagent.

(8) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

(1) Biotek EL 301 Microwell Reader, Awareness Technology Inc. Stat-Fax Model 303 PLUS, or Hyperion MicroReader™ 3 Model No. 4027-002 with 450-nm filters.

(2) RIDA™SOFT Win Software.

(3) 50 µl, 100 µl, and 1000 µl Pipettor and pipette tips.

(4) Graduated cylinders (plastic or glass): 100 ml, 1 liter.

(5) Sample shaker (optional).

(6) Filter funnel.

(7) Whatman #1 filter paper or equivalent.

(8) Balance.

(9) Stepper pipetter.

(10) Paper towels, Kaydry paper or equivalent absorbent material.

(11) Waste receptacle.

(12) Timer: 3 channel minimum.

(13) Waterproof marker, Sharpie or equivalent.

(14) Wash bottle.

(15) Deionized or distilled water.

10.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

When stored properly (at 39º F) the Wash Solution has a shelf life of four weeks.